

Application Serial No. 10/655,874
Amendment and Response dated March 14, 2005
Response to Office Action of September 13, 2004

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1. (currently amended) A process for extracting a heterologous target protein from *E. Escherichia coli* cells, comprising the following steps:

- a) lowering the pH of a solution containing whole *E. Escherichia coli* cells expressing said a heterologous target protein to form an acidic cell solution;
- b) adding at least one solubility enhancer to the solution containing the
Escherichia coli cells;
- b) c) disrupting the cells to release the heterologous target protein into the acidic solution; and
- e) d) separating cellular debris from the released heterologous target protein to obtain a protein preparation product enriched in the heterologous target protein.

Claim 2. (original) The process of claim 1, wherein the pH is lowered to a pH of about 4.0 to about 5.0.

Claim 3. (cancelled)

Claim 4. (original) The process of claim 1, wherein the pH is lowered to no more than 4.0.

Claim 5. (currently amended) The process of claim 1, wherein said separating step comprises centrifugation.

Claim 6. (currently amended) The process of claim 5, further comprising purifying the heterologous target protein from the protein preparation product.

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Claim 7. (original) The process of claim 6, wherein said purifying comprises column chromatography.

Claim 8. (original) The process of claim 7, wherein said column chromatography is expanded bed chromatography.

Claim 9. (cancelled)

Claim 10. (currently amended) The process of claim 1 9, wherein said at least one solubility enhancer is added to the solution prior to said pH lowering in step a).

Claim 11. (currently amended) The process of claim 1 9, wherein said at least one solubility enhancer is added to the solution ~~prior to or contemporaneous~~ contemporaneously with said pH lowering in step a).

Claim 12. (currently amended) The process of claim 1 9, wherein the at least one solubility enhancer comprises a divalent cation.

Claim 13. (original) The process of claim 12, wherein the divalent cation comprises magnesium or calcium.

Claim 14. (currently amended) The process of claim 1 9, wherein the at least one solubility enhancer is polyethyleneimine (PEI).

Claim 15. (currently amended) The process of claim 1 9, wherein the step b) comprises adding PEI and the at least one solubility enhancer comprises a divalent cation and PEI.

Claim 16. (original) The process of claim 12, wherein the divalent cation is added at a concentration of about 10 mM to about 150 mM.

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Claim 17. (original) The process of claim 14, wherein the PEI is added at a concentration of about 0.2% to about 0.3% vol/vol of a 50% wt/vol solution.

Claim 18. (cancelled)

Claim 19. (withdrawn) A method for decreasing biomass-biomass interactions, biomass-resin interactions, or a combination thereof, of a solution of disrupted *E. coli* cells, comprising:

- a) lowering the pH of a solution containing whole *E. coli* cells expressing a heterologous target protein to form an acidic cell solution; and
- b) disrupting the cells to release protein into the acidic solution; wherein the biomass-biomass interactions, biomass-resin interactions, or a combination thereof of the disrupted cell solution is reduced as compared with a solution of cells disrupted at a non-acidic pH.

Claim 20. (withdrawn) The method of claim 19, wherein the pH is lowered to about 4.0 - 5.0.

Claim 21. (withdrawn) The method of claim 20, wherein the pH is lowered to about 4.0 - 4.5.

Claim 22. (withdrawn) The method of claim 21, wherein the pH is lowered to no more than 4.0.

Claim 23. (withdrawn) The method of claim 19, further comprising adding at least one solubility enhancer to the *E. coli* cell solution.

Claim 24. (withdrawn) The method of claim 23, wherein the at least one solubility enhancer comprises a divalent cation.

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Claim 25. (withdrawn) The method of claim 24, wherein the divalent cation comprises magnesium or calcium.

Claim 26. (withdrawn) The method of claim 23, wherein the at least one solubility enhancer is PEI.

Claim 27. (withdrawn) The method of claim 23, wherein the at least one solubility enhancer comprises a divalent cation and PEI.

Claim 28. (withdrawn) The method of claim 24, wherein the divalent cation is added at a concentration of about 10 mM to about 150 mM.

Claim 29. (withdrawn) The method of claim 26, wherein the PEI is added at a concentration of about 0.2% to about 0.3% vol/vol of a 50% wt/vol solution.

Claim 30. (withdrawn) A method for altering a flocculent in a solution of disrupted *E. coli* cells, comprising:

- a) lowering the pH of a solution containing whole *E. coli* cells expressing a heterologous target protein to form an acidic cell solution;
 - b) disrupting the cells to release protein into the acidic solution;
- wherein moisture content of a flocculent in the released protein solution is greater when cells are disrupted in an acidic solution as compared with a non-acidic solution.

Claim 31. (withdrawn) The method of claim 30, wherein the pH is lowered to about 4.0 - 5.0.

Claim 32. (withdrawn) The method of claim 31, wherein the pH is lowered to about 4.0 - 4.5.

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Claim 33. (withdrawn) The method of claim 32, wherein the pH is lowered to no more than 4.0.

Claim 34. (withdrawn) The method of claim 30, further comprising adding at least one solubility enhancer to the *E. coli* cell solution.

Claim 35. (withdrawn) The method of claim 34, wherein the at least one solubility enhancer comprises a divalent cation.

Claim 36. (withdrawn) The method of claim 35, wherein the at least one divalent cation is magnesium or calcium.

Claim 37. (withdrawn) The method of claim 34, wherein the at least one solubility enhancer is PEI.

Claim 38. (withdrawn) The method of claim 34, wherein the at least one solubility enhancer comprises a divalent cation and PEI.

Claim 39. (withdrawn) The method of claim 35, wherein the divalent cation is added at a concentration of about 10 mM to about 150 mM.

Claim 40. (withdrawn) The method of claim 37, wherein the PEI is added at a concentration of about 0.2% to about 0.3% vol/vol of a 50% wt/vol solution.

Claim 41. (withdrawn) A protein product produced by the process of claim 1.

Claim 42. (withdrawn) A protein product produced by the method of claim 19.

Claim 43. (withdrawn) A protein product produced by the method of claim 30.